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143. Immunological and functional properties of two monoclonal antibodies against human C5

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Two monoclonal antibodies designated 4C6 and 3B2 were produced by immunizing Balb/c (4C6) or DBA, mice (3B2) respectively with human C5 purified according to DESSAUER et al. Immunobiol., in press) and following the fusion of the mouse spleen cells with NS-1 (4C6) or X63.Ag8.653 (3B2) lymphoma cells respectively. Hybridomas have been selected according to the results of ELISA and RIA measurements with insolubilized human C5. They were cloned and IgG fractions of ascites were isolated by 45 % saturated ammonium sulfate precipitation and DEAE ion exchange chromatography. These fractions were investigated by analytical and functional assays.

3B2

The antibodies (ab) in one of the lines (4C6) reacted with a 200 kD protein after SDS-PAGE and immunoblotting (IB) of unreduced human C5, whereas line 3B2 reacted with a 60 kD peptide chain, which is suspected to represent a split product of C5, since its proportion increased with time during the storage of C5. Line 4C6 failed to react with reduced C5 in the IB analysis, in contrast line 3B2 reacted even after reduction of C5 with the 60 kD band. Functionally this antibody (3B2) inhibited the ^{3}H serotonin release from guinea pig platelets induced by hog C5a and surprisingly also the lysis of chicken erythrocytes by C56 + C7, C8, C9 and the lysis of EAC1–5 by C6–9 or EAC1–6 by C7–9, but failed to inhibit the interaction of C5 with EAC1423 in the presence of C6–9 in excess. The other antibody (4C6), after preincubation with C5 strongly inhibited the C5 dependent lytic activity when studied with EAC1423 + C6–9 and also diminished the lysis of ChE by C56 and C7–9, but failed to react with EAC1–6.

Our findings suggest that one of the monoclonal ab (4C6) detects an epitope on the C5 molecule related to its reaction with target membranes. This epitope seems to be hidden or without further functional role on EAC1–5 or EAC1–6.

The other monoclonal ab (3B2) displaying functional inhibitory activities puzzles by its functional inhibitory effect on C5a dependent serotonin release on one side and its reaction with cell-bound C5 or free and bound C5b6. Further studies are needed to clarify, whether these contradictory results are due to the possibility, that C5a is produced by cleavage but remains noncovalently bound to C5b.

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144. Potentiation of antibody-dependent cellular cytotoxicity and chemiluminescence in human neutrophils by platelet activating factor

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Human neutrophils (PMN) purified by elutriation from blood of healthy volunteers were tested in the presence of platelet activating factor (PAF) for both antibody-dependent cellular cytotoxicity (ADCC) against antibody coated erythrocytes and the oxidative response as

250 ug/ml
100 ug/ml